

Benthic species assemblages change through a freshwater cavern-type cenote in the Yucatán Peninsula, Mexico

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Abstract

We studied benthic assemblages through X-Batún, a continental freshwater cenote and its associated submerged cave located in San Antonio Mulix (Yucatán, Mexico). Using cave diving techniques, we collected sediment samples at four zones of the system. We extracted and counted individuals of benthic species in three replicates of 5 grams of wet sediment at each site. The biological composition was integrated by 15 species from eight higher taxonomic groups. Non-metric multidimensional scaling distinguished four assemblages that coincided with surface, open water, cavern and cave zones. ANOSIM test revealed significant differences between the assemblages. In the deeper zones of the cenote characterized by twilight and total darkness, Ostracoda and Gastropoda show the highest diversity and abundance, with practical absence in surface sediments. This pattern may suggest ecological interactions with chemosynthetic bacterial activity. Surface shows an assemblage typical of epigeal environments. Environmental variables along the cenote varied little from the upper layers to bottom. Linear correlation and detrended canonical analysis revealed that light is the main driver of benthic species assemblages. Temperature, pH, and dissolved oxygen exert higher influence at individual biological benthic assemblage in X-Batún.

Keywords

benthic assemblages, cenote, groundwater, light gradient, Ostracoda, zonation

Introduction

Yucatán is a karstic platform composed primarily in Cenozoic limestone which was formed mainly from coral reefs (Marín et al. 2000; Pacheco and Alonzo 2003). One of the main characteristics of the hydrogeology of Yucatán is the presence of “cenotes”: water-filled dolines formed by the dissolution and collapse of limestone rock (Gaona-Vizcaino et al. 1980). Cenotes are highly heterogenous regarding their geomorphology, given distinct paths of dissolution and diagenesis of carbonate (MacSwiney et al. 2007; Brankovits et al. 2021). Most cenotes are, therefore, unique in shape and structure, with highly variable internal features such as subterranean passages, caves, conduits, and display different degrees of interaction with marine and terrestrial environments. The high availability of ecological niches and habitats within cenotes provides conditions for high levels of endemism and complex ecological interactions for both, terrestrial and aquatic taxa (Calderón-Gutiérrez et al. 2018; Chávez-Solís et al. 2020). In fresh and anchialine environments of the Peninsula, for example, about ~30 stygobiont (obligate aquatic subterranean) endemic malacostracan species have been recorded so far (Angyal et al. 2020).

In subterranean waters, biological composition is known to be primarily structured by environmental gradients (Sánchez et al. 2002; Pellegrini et al. 2018). Bulk organic matter, water quality and sun light are key for species diversity and distributions (Martí et al. 2004; Brankovits et al. 2017; Bishop et al. 2020).

The general view of the aquatic biodiversity structure in subterranean waters suggests that abundance and diversity of organisms progressively decrease from the highly populated and energy-rich well illuminated zones to the energy-deprived and poorly populated deeper zones (Mammola 2019). This assumption is supported by the fact that well-illuminated zones of the cenotes, usually known as “windows to the surface”, are the regions where primary production by photosynthetic organisms happens and where allochthonous organic material enter to the subterranean system (MacSwiney et al. 2007; Hendus et al. 2019). However, there is increasing evidence that food sources originated from bacterial communities and nutrient transport are relevant for supporting trophic web chains in zones where sun light is limited or totally lacking (Brankovits et al. 2017; Chávez-Solís et al. 2020). Benthic assemblage structure along subterranean waters can therefore significantly vary from site to site, even within the same region (Rodríguez 2002; Cresswell 2019; Borisov et al. 2021).

Several publications deal with the characterization of the stygobiont fauna that inhabits the water column and the associated microhabitats (such as the surface of rocks or organic material deposits) of the Yucatán cenotes and their caves (eg. Álvarez et al. 2015; Espinasa et al. 2019; Angyal et al. 2020; Liévano-Beltrán and Simoes 2021), but only scarce information is available about the benthic communities of the Yucatán aquifer complex.

Smirnov and Elías-Gutiérrez (2010) investigated the biocenotic characteristics of 25 cenotes, lagoons, and wetlands of the Yucatán Peninsula, which concluded that among the invertebrate remains, ostracodes were the most common, as they were absent only in four of the studied freshwater bodies. Grego et al. (2019) published

new distribution records and new species of freshwater subterranean gastropods from benthic samples of some continental cenotes in the eastern part of Yucatán state and found three species of which two were new to science. Except these efforts, despite the enormous volume of the Yucatán cenote aquifer, no systematic and ecological studies of the cave benthic fauna had been done so far and key questions about benthic communities remain unanswered, such as how benthic fauna are structured along the Yucatán Peninsula tropical subterranean aquifer? what are the ecological interactions established between biotic and abiotic components? and how community is affected at regional scale by anthropogenic activities developed in surface?

In this study, we characterize the benthic assemblages, based on invertebrate and other remains of the cenote X-Batún, and evaluate how these assemblages vary along four well-differentiated zones of the cenote. We collected information about the variation of the physical and chemical parameters of the water column continuously along the cave system, allowing explanation of how the distribution of benthic invertebrates could be determined by environmental characteristics.

Methods

Study area

The study system was cenote X-Batún in San Antonio Mulix, Umán, Yucatán, México (20°40'26.6"N, 89°46'22.1"W) (Fig. 1). This cenote is part of the Ring of Cenotes, a circular fracture zone with high density of sinkholes identified as the outer rim of the crater where a meteorite impacted the peninsula 66 million years ago (Bauer-Gottwein et al. 2011).

Cenote X-Batún is an oligotrophic freshwater cenote open for the tourism, characterized by a fully solar-irradiated open water pool covered with dense vegetation. X-Batún surficial waters are dominated by aquatic rhizomatous herbaceous perennials (Nymphaeaceae). The system is characterized by a water-filled cavern in the twilight zone with fragmented rock slopes covered with decaying organic material, and with two submerged cave passages in the dark zone. In Yucatán Peninsula, three seasons occur: a dry season with high temperatures and low rainfall of about 400 mm yr⁻¹ during March to May, a rainy season with frequent rainfall with values increasing from 900 to 1400 mm yr⁻¹ takes place from June to October, and a cold-fronts season with winter storms and occasional rainfall from November to February (Pérez-Ceballos et al. 2012).

Sampling procedure, specimen sorting and identification

A sampling campaign was undertaken on 25 May of 2021 by Cenoteando team (Universidad Nacional Autónoma de México, UMDI-F. Ciencias-Sisal, <https://www.cenoteando.mx/>). This campaign was authorized by the Secretaría de Desarrollo Sustentable del Estado de Yucatán, Dirección de Gestión y Conservación de Recursos Naturales with authorization

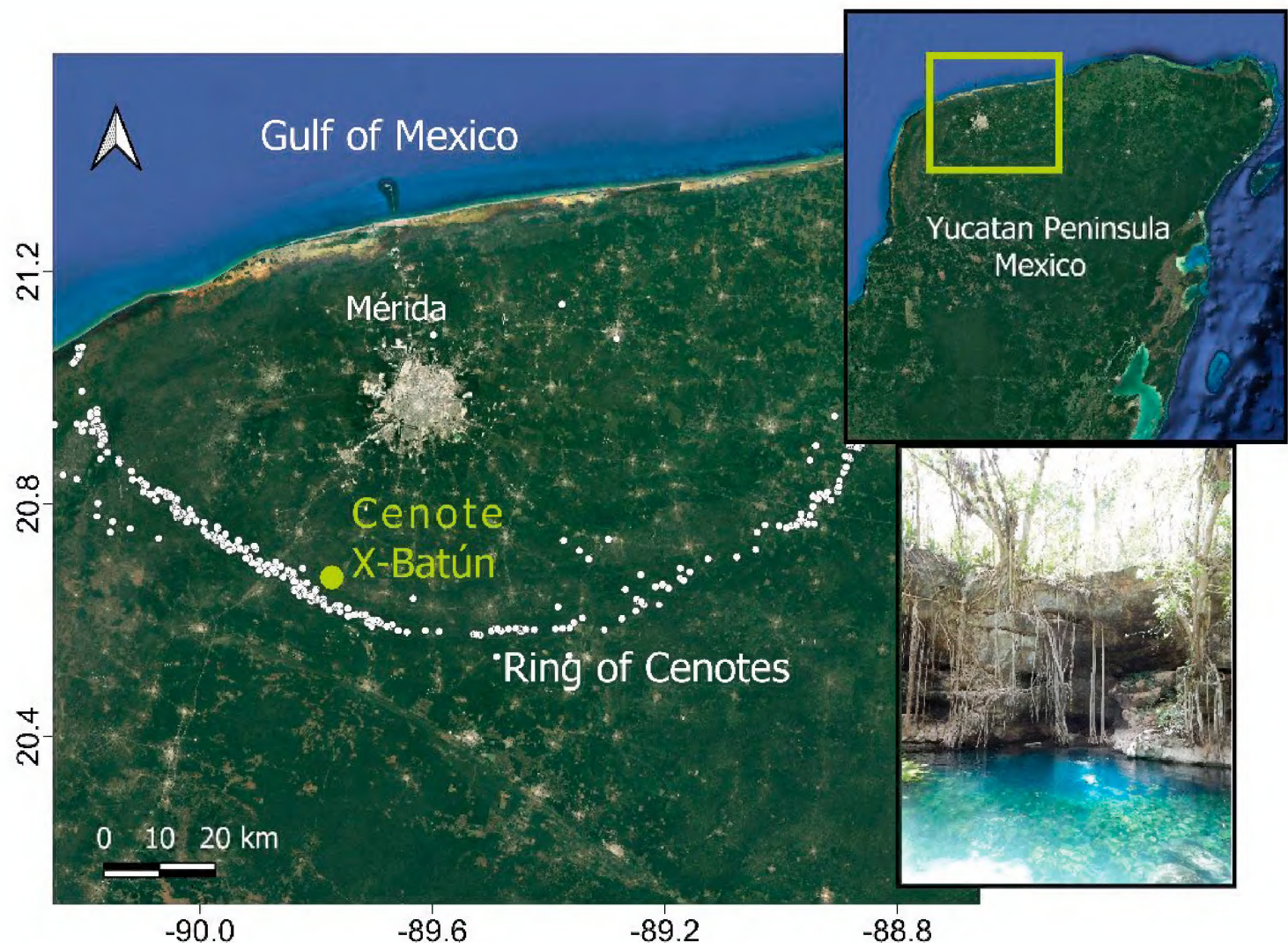


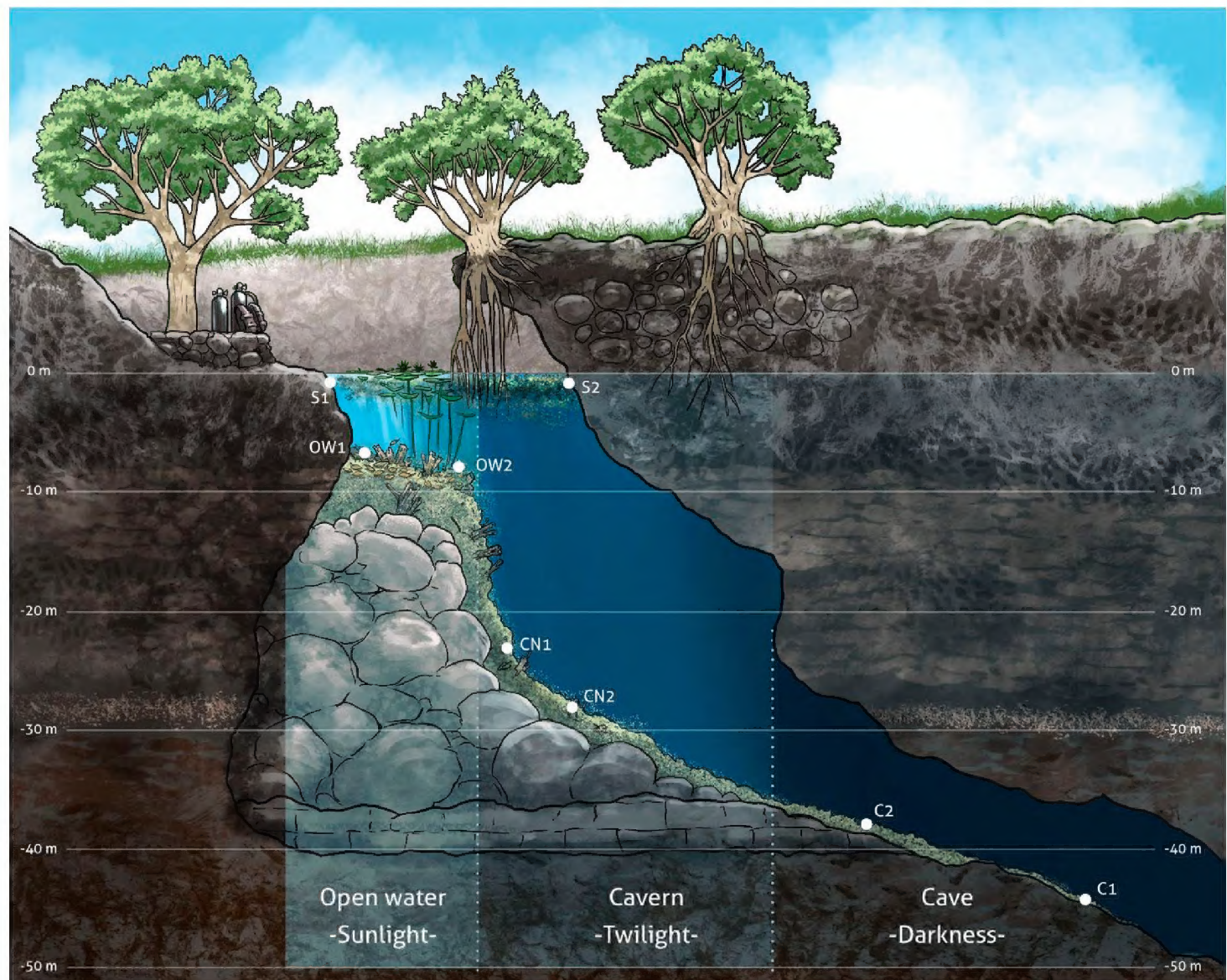
Figure 1. Location of the studied cenote in the Yucatán Peninsula. Yellow dot illustrates the geographical position of Cenote X-Batún within the Ring of Cenotes.

number VI-0884-2021. Using scuba diving techniques, we collected sediment samples at four zones of the cenote. Three of the four examined zones were defined and adapted from Chávez-Solís et al. (2020), as follows: “water surface” (S) is the uppermost, fully illuminated water layer with photosensitizing partially submerged aquatic vegetation, algal communities and with aquatic fauna typical of surface waters. “Open water” (OW) or “cenote pool” is a region under the water surface that receives direct influence from the surroundings i.e., the air interface, organic debris, interaction with animals from the surface and, in some cases, direct or indirect sun light. The “cavern” (CN) is the transition zone from the open water to the cave: a region where sunlight is still perceivable but has no direct incidence, there is less external influence and has no vertical access to the surface. The “cave” (C) is the aphotic region, with minimum or no influence from external factors and no vertical access to the surface (Table 1, Fig. 2). At each of these four zones, two sediment samples were collected using plastic Ziplock bags. Sediments were taken only from the uppermost three centimeters of the cenote floor to capture only recent biological material (not fossil or subfossil). Final weigh at each bag was about 2 kg of wet sediment.

Specimen extraction and counting were carried out in three replicates of 5 grams of wet sediment at each of the samples collected. We used a stereomicroscope for specimen sorting and those with soft parts, complete shells or well-preserved valves were extracted and counted as individuals. In case of ostracodes, to avoid over representation,

Table 1. Data of the sampling sites and characteristics of sediment samples collected from four zones of cenote X-Batún. DOM = decaying organic matter.

Sample code	Hydro-zones	Illumination	Depth	Characteristics
S1	water surface	Sunlight	<1.0 m	thin layer of sediment composed by algae and DOM
S2	water surface	Sunlight	<1.0 m	thin layer of sediment composed by algae and DOM
OW1	open water	Sunlight	-7.1 m	thick layer of sediment, lot of DOM
OW2	open water	Sunlight	-7.5 m	thick layer of sediment, lot of DOM
CN1	cavern	Twilight	-24.0 m	thick layer of sediment, lot of DOM
CN2	cavern	Twilight	-27.7 m	thinner layer of sediment than at sampling site CN1, less DOM
C1	cave	Darkness	-44.3 m	very thin layer of sediment covered with calcite crystal plates, DOM not visible
C2	cave	Darkness	-37.7 m	thicker layer of sediment than at sampling site C1, DOM not visible

**Figure 2.** Schematic drawing of Cenote X-Batún with the four different zones of the cenote evaluated, and sampling sites in the sediment layer. S1 = water surface 1, S2 = water surface 2, OW1 = open water 1, OW2 = open water 2, CN1 = cavern 1, CN2 = cavern 2, C1 = cave 1, C2 = cave 2.

we counted the right valve only, when free valves were recovered. For each zone of the cenote, species average values were calculated. Individuals were deposited in Eppendorf tubes with 96% ethanol and are available in the collection of ostracodes of the Tecnológico Nacional de México – I. T. Chetumal.

Specimen identifications were carried out with invertebrate specialized literature (e.g. Meisch 2000; Karanovic 2012 for ostracodes; Grego et al. 2019 for gastropods) and if possible, by consulting taxonomists with experience on invertebrate aquatic tropical taxa. Representative individuals of each of the taxonomic groups found in the cenote were photographed with an Olympus BX51 microscope. Using the photo stacking method and the weighted averaging algorithm in Helicon focus 7.6.6 software, we obtained final photographs that show specimen details focused (Fig. 3).

Environmental variables were measured in situ using an YSI Exo 2 Multiparameter probe (made in USA, range 0 to 200 mS/cm, respond T63<2seg, resolution 0.0001 to 0.01 mS/cm, type of sensor nickel cell 4 electrodes). Profiles of temperature, pH, total dissolved solids (TDS), conductivity and dissolved oxygen were obtained. Along the water column, measurements were taken every 2 cm, and more than a thousand values were recorded for each variable from the top to deepest bottom of the cave. The resulting database was manually edited by eliminating those records duplicated by depth. Final dataset consisted in 350 records.

Statistical analysis

Benthic assemblage distribution through cenote X-Batún

We determined ecological attributes of the evaluated zones of the cenote using the following diversity metrics: species richness, evenness and α diversity, using Shannon diversity index. The ANOVA test was used to determine statistical differences in the diversity between the zones of the cenote.

Benthic species associations along the Cenote X-Batún were determined with non-metric multidimensional scaling (NMDS). The NMDS generates an ordination in a two-dimensional space, representing the pairwise dissimilarity between species according to their occurrences. We first calculated the Bray-Curtis dissimilarity coefficient, using total abundance data, previously transformed by square root (Clarke and Warwick 1994).

Analysis of similarities (ANOSIM) (Clarke 1993) was then used to identify significant differences in composition and abundance of benthic assemblages between the zones defined in the NMDS analysis. To test the statistical contribution (%) of each species to the composition and abundance similarity determined in the Bray-Curtis matrix we used the SIMPER analysis. Statistical analyses were conducted in the software package PRIMER v6 (Clarke and Gorley 2006).

To estimate the relative importance of the environmental variables measured along the cenote associated with the species assemblage structure, we performed a linear correlation test (Pearson correlation).

Detrended Correspondence Analysis (DCA) with detrending by segments and non-linear rescaling was conducted to estimate major gradients in species composition using the CANOCO version 5 package (Šmilauer and Lepš 2014). The abundance data were logarithmically transformed, and rare species were down weighted. Ordination site scores were correlated to medians and coefficient of variation of all

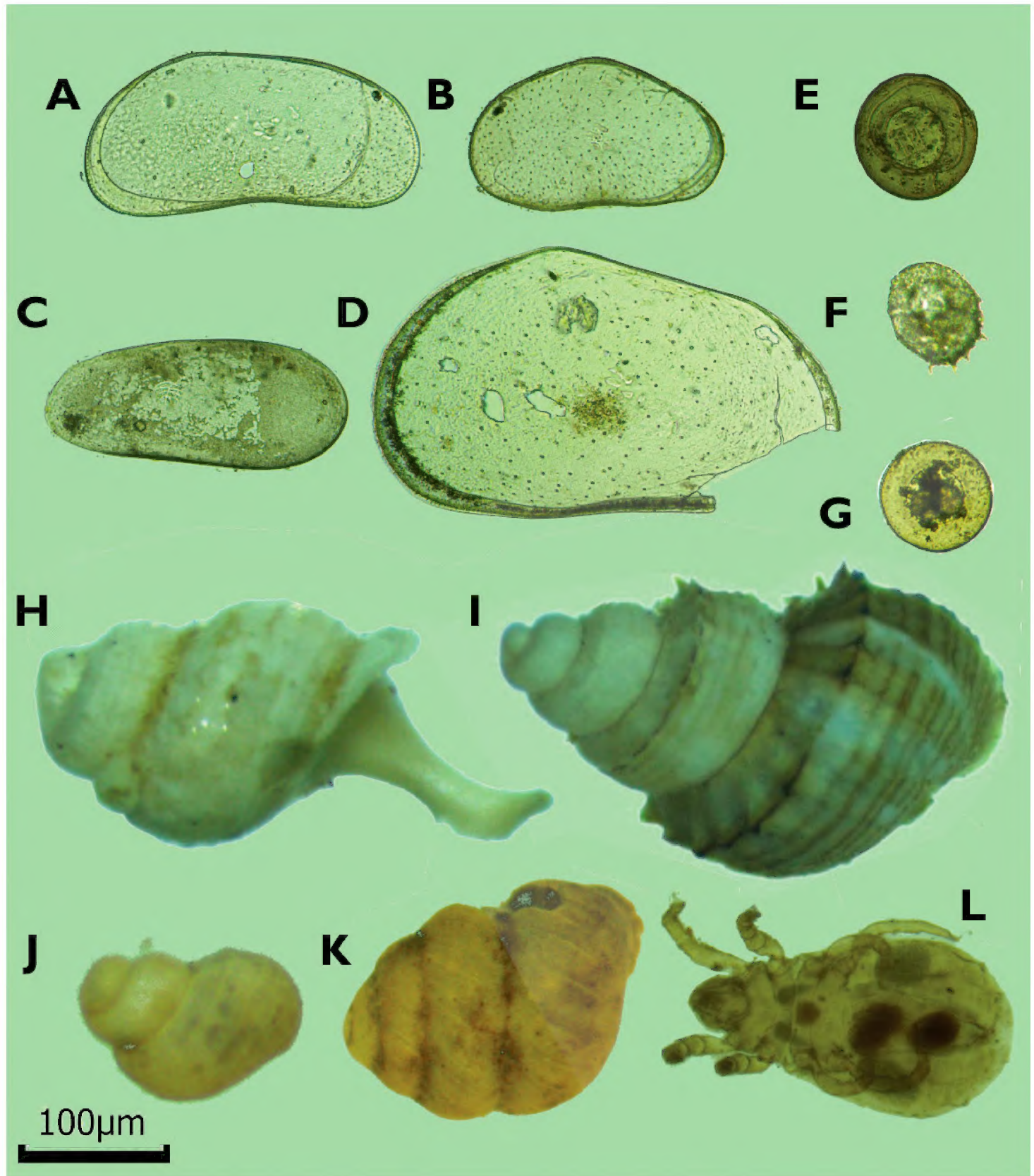


Figure 3. Benthic taxa of sediment samples extracted from Cenote X-Batún **A** *Pseudocandona* sp. **B** *Cypridopsis vidua* (Müller, 1776) **C** *Darwinula stevensoni* (Brady & Robertson, 1870) **D** Cyprididae sp. **E, G** *Arcella megastoma* (Penard, 1902) **F** *Centropyxis discoides* (Penard, 1890) **H–J** *Pyrgophorus coronatus* (L. Pfeiffer, 1840 **K** *Bothriopupa* sp. **L** *Tyrphonotheus* sp.

measured environmental factors by means of Kendall tau. Medians and coefficients of variation of all environmental variables were plotted onto DCA ordination diagram as supplementary environmental data for ecological interpretations.

Environmental variables were standardized and added by forward selection using the Monte Carlo permutation test with 999 permutations ($\alpha = 0.05$). Calculations and final ordination graphs of the DCA were performed using the software Canoco version 5 (Šmilauer and Lepš 2014).

Results

Species diversity metrics in Cenote X-Batún

A total of 15 aquatic species belonging to eight higher taxonomic groups were found in the sediments of Cenote X-Batún. The main groups identified are foraminifers, testate amoebae, bivalves, gastropods, copepods, ostracodes, mites and insects. We identified the specimens down to genus level and five of them were also identified to species level.

Species richness was relatively homogeneous throughout the cenote with values ranging from seven to eight species at each zone evaluated, but most zones display a unique species composition (Fig. 4). Ostracodes display the highest species richness with four species.

In terms of abundance, ostracodes were also the most abundant group in the cenote. The species *Darwinula stevensoni* displayed the highest abundance with 98 ind g⁻¹, followed by testate amoeba *Arcella megastoma* (68 ind g⁻¹), the ostracod *Cypridopsis vidua* (46 ind g⁻¹) and the gastropod *Pyrgophorus coronatus* (45 ind g⁻¹). Alpha diversity based on Shannon index values range from 1.1 to 1.84, revealing relatively low species diversity in the system. The ANOVA test reveal that there are not significant differences between Shannon diversity index at each zone evaluated ($F(3,4) = 3.07$, $p = 0.15$), and therefore, diversity can be considered homogeneous along the cenote. Evenness was highly variable ranging from 0.43 to 0.9, suggesting differences in species total abundances between sites. The open water zones display almost homogeneous abundances among their species, whereas surface and cave zones display, variable abundances between their respective species (Fig. 4).

The benthic assemblages were not continuously distributed along the cenote (Table 2). Ostracodes and gastropods were mostly distributed toward the deepest zones of the cave, which is characterized by less light availability. Chironomids, testate amoeba and copepods were distributed mainly toward the surface.

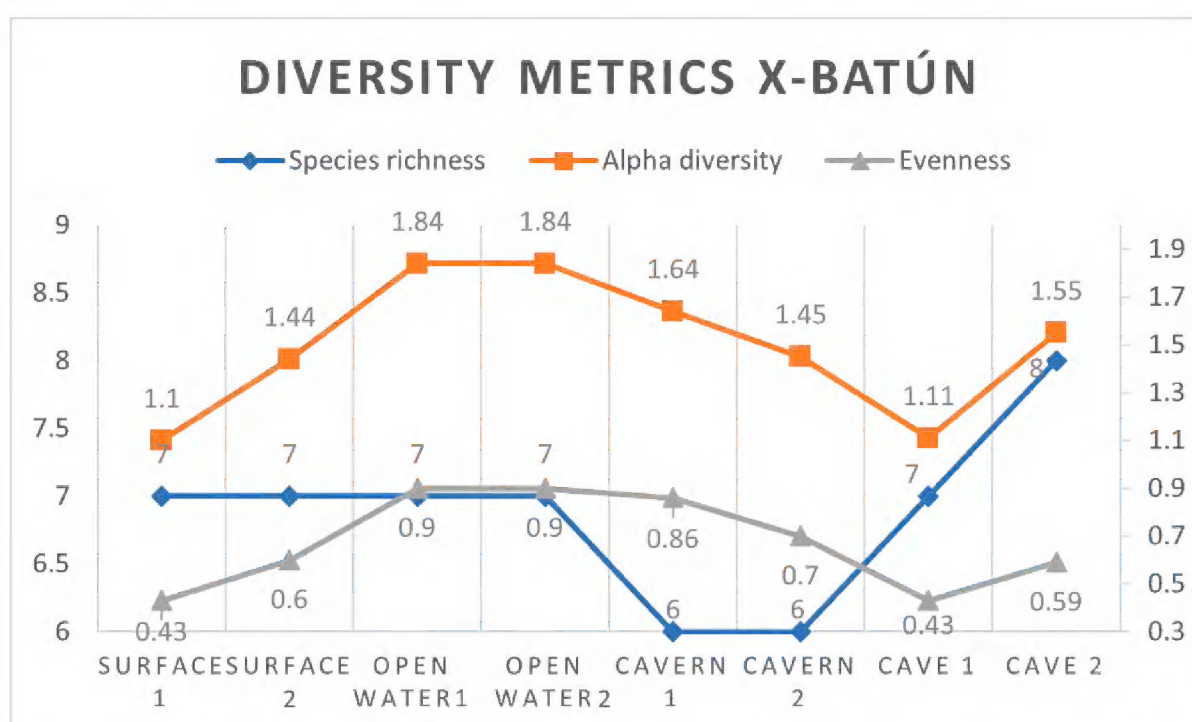


Figure 4. Benthic fauna diversity metrics along an environmental gradient in cenote X-Batún.

Four different assemblages were discriminated in the NMDS ordination with 70% of Bray-Curtis similarity (Fig. 5). The assemblage G1 coincided with water surface zone and was constituted by testate amoeba, chironomids, mites and copepods. The assemblage G2 was integrated by testate amoeba, gastropods and ostracodes. All from open water zone of the cenote. Assemblages G3 and G4 were constituted by cavern and cave sites, respectively. On these two zones of the cenote, ostracodes and gastropods were the species better represented.

Table 2. Occurrence of the benthic taxa extracted from eight sediment samples of four zones of Cenote X-Batún. S1 = water surface 1, S2 = water surface 2, OW1 = open water 1, OW2 = open water 2, CN1 = cavern 1, CN2 = cavern 2, C1 = cave 1, C2 = cave 2. + = presence, – = absence.

Taxa	Higher taxon	S1	S2	OW1	OW2	CN1	CN2	C1	C2
Foraminifer	Foraminifera	–	–	–	–	–	+	–	–
<i>Arcella megastoma</i>	Protozoa	+	+	+	+	+	–	+	–
<i>Centropyxis aculeata</i>	Protozoa	+	+	+	+	–	–	–	–
Bivalve	Bivalvia	–	–	–	–	–	–	–	+
<i>Pyrgophorus coronatus</i>	Gastropoda	–	–	+	+	+	+	+	+
<i>Bothriopupa</i> sp.	Gastropoda	–	–	–	–	+	+	+	+
Copepod	Copepoda	+	+	–	–	–	–	–	–
<i>Darwinula stevensoni</i>	Ostracoda	–	–	+	+	+	+	+	+
<i>Cypridopsis vidua</i>	Ostracoda	–	–	+	+	+	+	+	+
<i>Pseudocandona</i> sp.	Ostracoda	–	–	–	–	–	–	–	+
Cyprididae sp.	Ostracoda	–	–	–	–	–	–	–	+
Hygrobatidae sp.	Acari	+	+	–	–	–	–	–	–
<i>Tyrphonotheirus</i> sp.	Acari	+	+	–	–	–	–	–	–
Chironomidae	Insecta	+	+	–	–	–	–	–	–

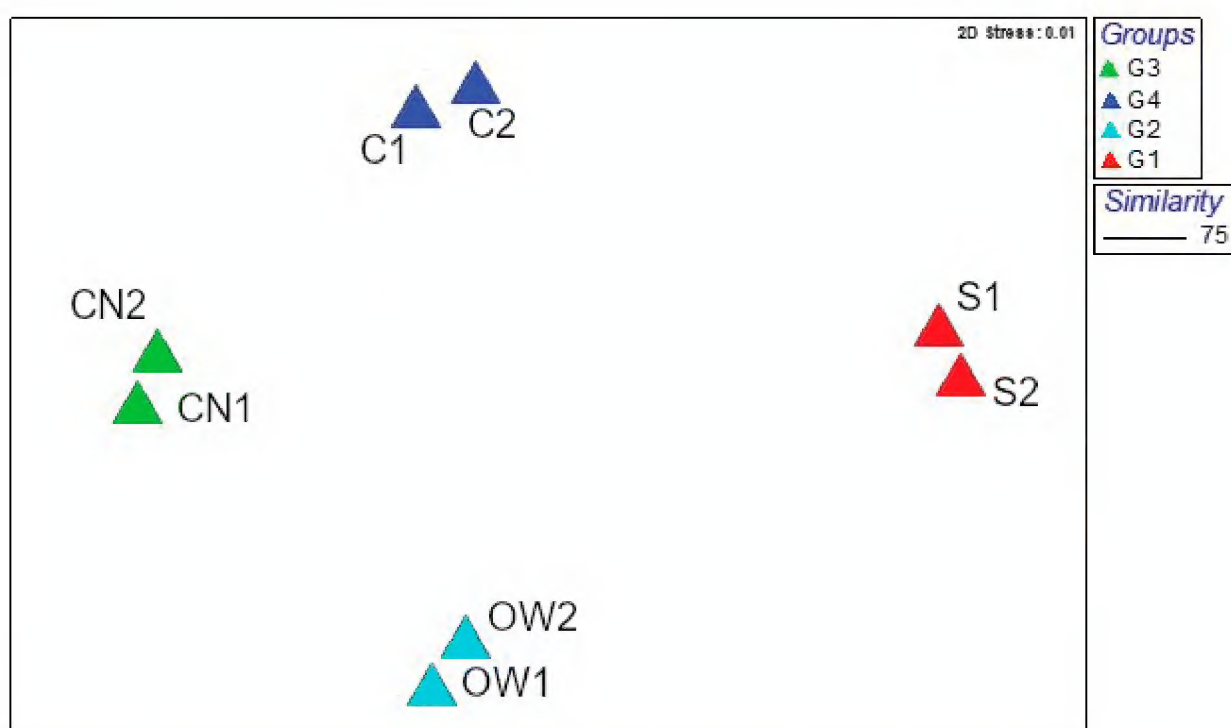


Figure 5. Non-metric multidimensional scaling plot based on Bray-Curtis index and squared root transformed abundance data of species from cenote X-Batún. Assemblage 1 (G1), assemblage 2 (G2), assemblage 3 (G3) and assemblage 4 (G4). Abbreviations are as follows: S – surface; OW – open water; CN – cavern; C – cave.

The SIMPER analysis was used to evaluate the contribution of each species to the Bray-Curtis associations. This analysis revealed that the taxa with higher contribution to the G1 assemblage, considering a cumulative percentage < 70%, were *Centropyxis aculeata*, *Arcella megastoma* y Chironomide. For G2 with a cumulative percentage of > 50%, *Cypridopsis vidua*, *Pyrgophorus coronatus* and *Bothriopupa* sp. were the most influential. For the G3 assemblage, *Darwinula stevensoni*, *Pyrgophorus coronatus* and *Cypridopsis vidua* were species with higher contribution with a cumulative percentage < 65%. On the G4 assemblage *Darwinula stevensoni*, and *Cypridopsis vidua* were the most influential (Table 3).

The ANOSIM test was then used to evaluate significant differences between the four habitat types identified in the NMDS. The ANOSIM test provides statistical support to determine that the four benthic assemblages are different in their composition and abundance ($R = 0.99$; $p < 0.001$). Ostracodes and gastropods for example, were mostly distributed toward the deepest zones of the cave, which is characterized by less light availability (Table 2). Chironomids, testate amoeba and copepods were distributed mainly toward the surface (Table 2).

Table 3. SIMPER analysis results, showing the percentage of contribution of the species for the four ecological groups discriminated in the non-metric multidimensional scaling analysis.

Grupos	Especies	% de Contribución	% acumulado
G1	<i>Centropyxis aculeata</i>	35.75	35.75
	<i>Arcella megastoma</i>	20.64	56.39
	<i>Chironomide</i>	13.06	69.45
G2	<i>Cypridopsis vidua</i>	19.79	19.79
	<i>Pyrgophorus coronatus</i>	17.90	37.70
	<i>Bothriopupa</i> sp.	13.34	51.04
G3	<i>Darwinula stevensoni</i>	26.05	26.05
	<i>Pyrgophorus coronatus</i>	20.17	46.22
	<i>Cypridopsis vidua</i>	16.47	62.69
G4	<i>Darwinula stevensoni</i>	39.38	39.38
	<i>Cypridopsis vidua</i>	21.53	60.91

Environmental variables

Vertical profiles of environmental variables at Cenote X-Batún show that conditions slightly change from surface to bottom, (Fig. 6). For temperature, the maximum variation is 0.5 °C, being the surface slightly warmer (27.9 °C) than bottom waters (27.4 °C). Similarly, pH displayed small changes along the water column, with overall neutral conditions and maximum variation of 0.05. TDS and conductivity show a similar behavior, with relatively low values at the first 10 meters and then progressively increasing to the bottom. For both variables, changes between surface and bottom conditions were relatively small. Dissolved oxygen shows a relatively small change from surface to bottom. Surface waters display about 5 mg/L, whereas bottom waters about 3.5 mg/L. In terms of dissolved oxygen availability, the entire system can be considered well oxygenated.

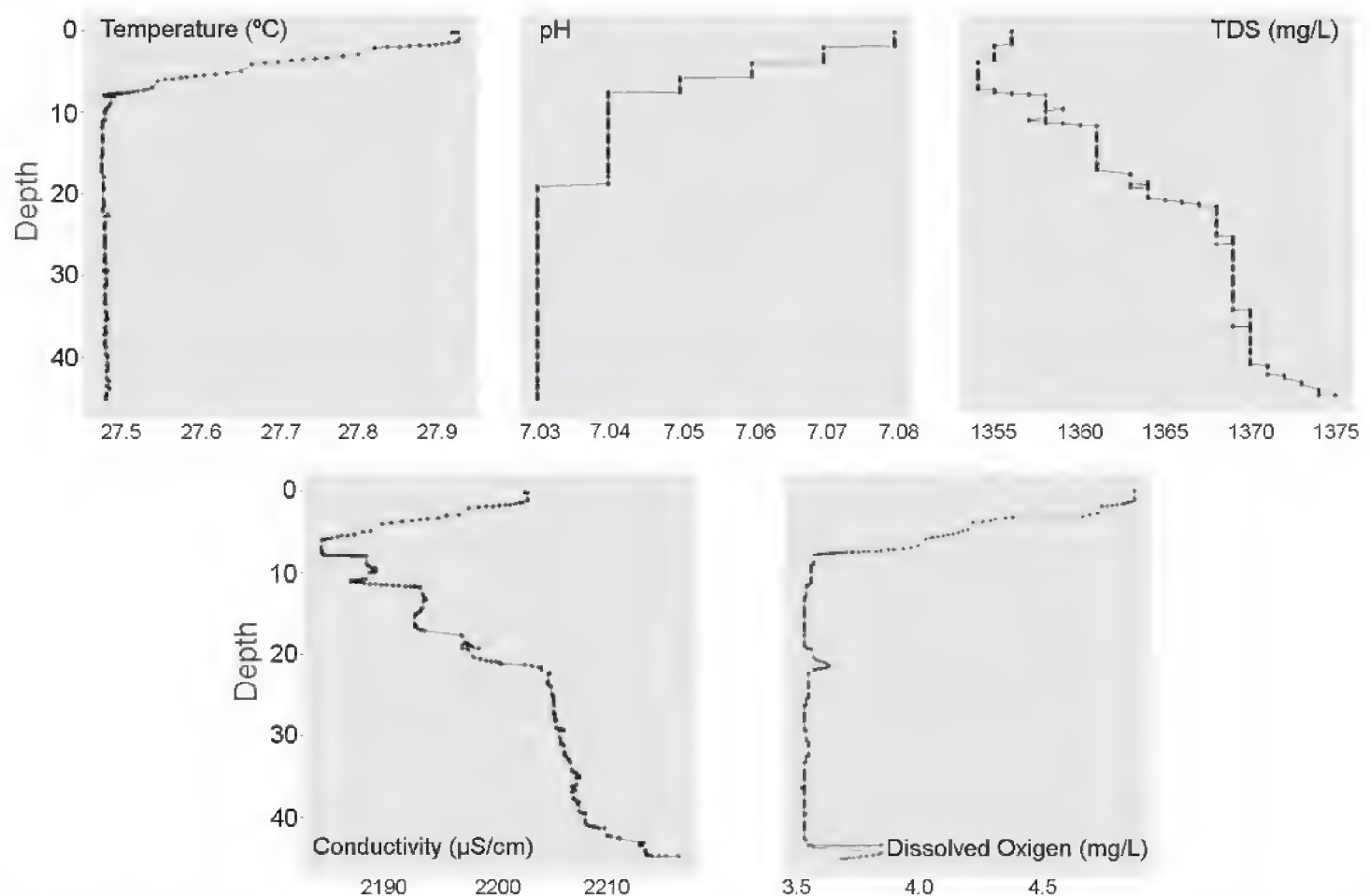


Figure 6. Water column environmental profiling of Cenote X-Batún: temperature, pH, total dissolved solids (TDS), conductivity and dissolved oxygen. Profiles are based on 350 measurements; black dots represent measured data.

Environmental influences on species assemblages of cenote X-Batún

Linear correlation demonstrated that light ($r = -0.98$, $p < 0.05$) and total dissolved solids ($r = -0.95$, $p < 0.05$) are more closely related to species assemblages determined in the NMDS.

The DCA, using environmental variables and species assemblages as supplementary variables, resulted in the following eigenvalues of the first two axes 0.76 and 0.08, respectively. Total inertia is 0.98. Cumulative percentage variance of species-environment for the first two axes are 76.1 and 84.9%. Kendall Tau correlation coefficient (significant at 0.01 level) shows that first DCA axis was strongly related to pH (-0.99^*), temperature (-0.97^*), dissolved oxygen (-0.95^*) and light (-0.84^*) were the explicative variables of the data set. In the DCA ordination plot, species assemblage of the outer zone (G1) of the cenote were positioned together with explicative variables of the first axis. The G2 assemblage was positioned close to the center of the axis revealing that environmental variables have limited effect on the species composition. The G3 and G4, corresponded to deeper zones of the cenote, are positively influenced by TDS and negatively influenced by variables such as DO, temperature, pH and light (Fig. 7).

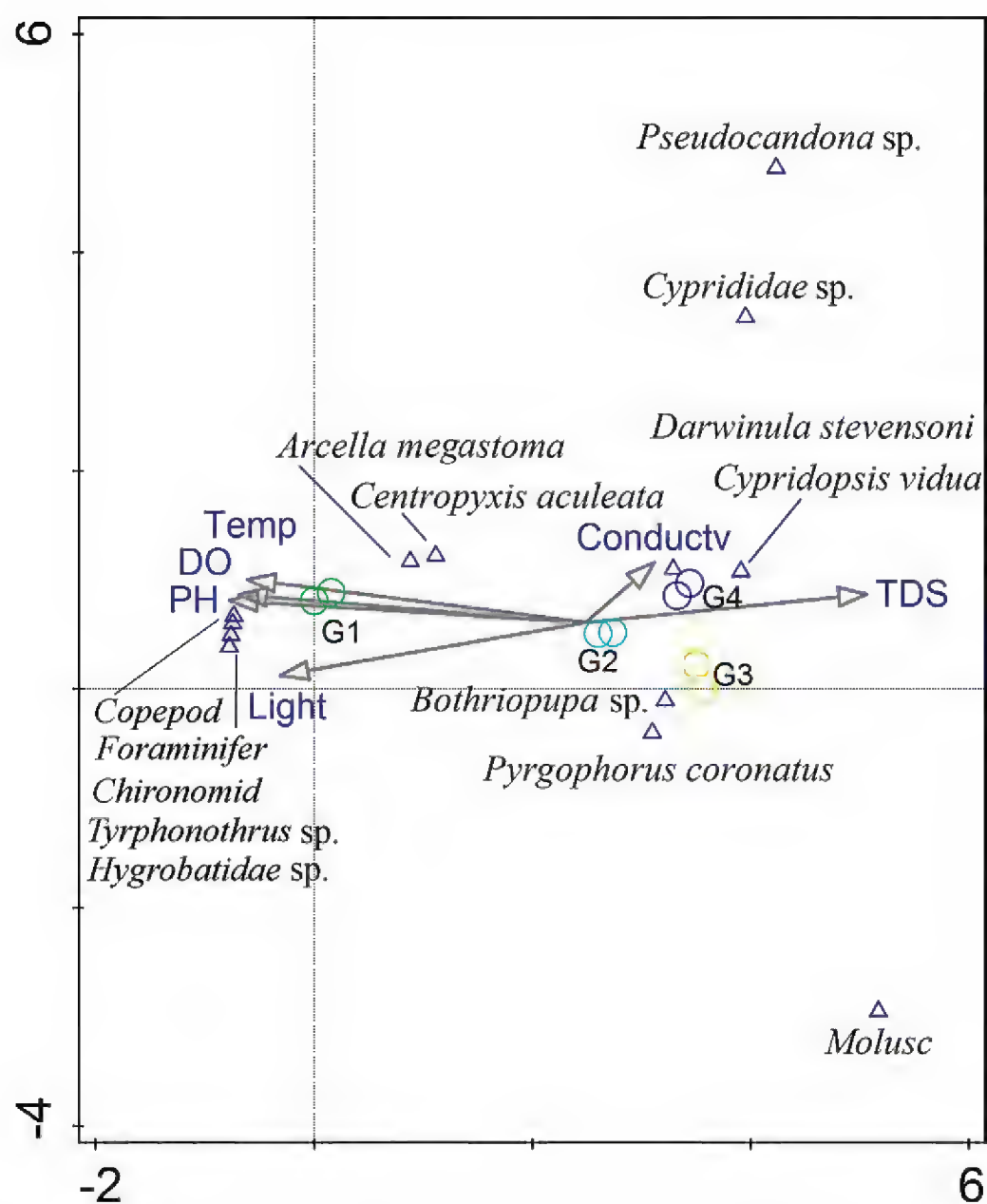


Figure 7. Detrended Correspondence Analysis (DCA) ordination diagram of benthic species and environmental variables in the cenote X-Batún. Statistical significance based on Monte Carlo test with forward selection $p \leq 0.05$. Abbreviations are as follows: temperature (Temp), dissolved oxygen (DO), conductivity (Conductv), total dissolved solids (TDS). G1 assemblage of surface sediments; G2 assemblage of open water sediments; G3 assemblage of cavern; G4 assemblage of cave sediments identified in NMDS. Colors corresponded to that in the NMDS plot.

Discussion

Benthic species assemblages of Cenote X-Batún

Benthic biological composition of Cenote X-Batún was integrated by eight higher taxonomic groups and 15 species. In terms of species richness and diversity, the cenote was relatively homogenous, as ANOVA test did not find significant differences among zones. Evenness index, however, suggest that zones of the cenote were variable in terms of species abundance. The fauna of X-Batún based on Shannon diversity index is relatively low, however, considering species richness of subterranean environments globally, the fauna is relatively high. In Križna jama cave system (Slovenia, 8km long), for example 32 troglobitic aquatic taxa have been recorded so far (Polak and Pipan 2021).

In the American continent, San Marcos artesian well and the Edwards aquifer (United States, 10500 km²) display 55 aquatic taxa (Hutchins et al. 2021) and Toca do Gonçalves cave (0.5km long) and Areias cave systems (14km long) in Brazil display 22 and 28, troglobitic/stygobitic taxa respectively (Souza and Ferreira 2016).

Ostracodes were the group in which we identified the highest number of species with four. All ostracod species and genera of X-Batún are previously known as epigean, as they are mainly distributed in lakes of this region (Cohuo et al. 2017), but in the cenote ecosystem they all inhabit the aphotic zone. Presence of epigean ostracod species in cenotes either with direct sun light incidence or cavern type with partial light or complete darkness, have been recently observed in the Yucatán Peninsula (Macario-González et al. 2021). First explanations of this phenomenon postulates that geologically recent increase of the water table in the Yucatán Peninsula, driven by the last deglacial, produced new habitats in subterranean systems (Yokoyama et al. 2000; Mueller et al. 2010; Cohuo et al. 2018), which presumably were occupied by epigean taxa. The species *D. stevensoni* and the genus *Pseudocandona* are the unique ostracod groups that have previously been observed in subterranean environments in the Yucatán Peninsula (Cohuo et al. 2017; Macario-González et al. 2021) and elsewhere in the world (Sohn 1983; Namiotko 2004; Namiotko and Danielopol 2004).

Gastropods are another group restricted to the aphotic zone in X-Batún. *Pyrgophorus* is a genus recently observed in photic and aphotic zones of cenotes of Yucatán, likely associated to the chemosynthetic production in aphotic zones (Grego et al. 2019).

Other species of X-Batún such as mites, testate amoebae (van Hengstum et al. 2008), chironomids (Vinogradova and Riss 2007; Pérez et al. 2013) and copepods (Suárez-Morales and Reid 2003) are mainly associated to surface waters. These species are commonly observed in lakes of the Yucatán Peninsula and mainly associated to high organic matter content (Pérez et al. 2013).

Benthic assemblages change along cenote X-Batún

The NMDS analysis, based on the biological composition, distinguished four assemblages, that coincided with the surface, open water, cavern, and cave zones of the cenote. The deepest zones of the cenote, corresponding to complete darkness sites, display the higher species abundance. The ANOSIM test revealed significant differences between the four species assemblages, suggesting that in fact the four zones are biologically distinct. This implies that benthic species assemblages are confined to specific zones along the light gradient in X-Batún. We disregard invertebrate postmortem sediment transportation along the cenote, as taphonomy of transported shells are characterized by shell edge or ornamentation modifications, abrasion, bioerosion, internal and external incrustations, and high percentage of totally or partially broken shells (Lazo 2006; Chattopadhyay and Das 2013). All these characteristics lacking in our samples.

The deeper zones of the cenote, cavern and cave, which are characterized by lack or limited sun light availability was constituted by three taxonomic benthic groups

and shown by the highest species abundance per gram of all sampling sites within the cenote. Ostracodes dominate the assemblage, the species *D. stevensoni* and *Cypridopsis* sp. show the higher statistical contribution to the assemblages as illustrated by the SIMPER analysis. Gastropods were also abundant in this zone almost equaling the abundance of *Cypridopsis* sp. Ostracodes are the animal group dominant in most sediment layers of epigeal environments such as lakes and cenotes of the Yucatán Peninsula (Smirnov and Elías-Gutiérrez 2011; Cohuo et al. 2017; Macario-González et al. 2021). Previous surveys highlighted that aquatic systems in this region, without ostracodes are rare (Cohuo et al. 2017). Cenote X-Batún was previously considered a system without ostracodes because the practical absence of living specimens or valve remains in surficial sediments. The high abundance of ostracodes and gastropods in the aphotic zone, and its practical absence in surficial environments, is therefore a remarkable finding.

Ostracodes are capable to feed on a variety of forms and resources such as algae, bacteria and some of them are carnivores or detritivores (Martens and Horne 2009). In the aphotic zone of X-Batún characterized by very thin layer of sediment, covered mainly with calcite crystal plates, is challenged to determine food source for specimens. Given cenote morphology with a slope of about 150° from the entrance to the cave zone we speculate that food source is not dependent of allochthonous organic material only, but from other sources such as chemoautotrophy. At global scale, in cave environments have been observed that chemical compounds such as hydrogen sulfide (H₂S) (Sarbu et al. 1996), methane and ammonium (Pohlman et al. 1997) can be synthesized by bacterial mats and transformed into organic products that become the main food source for subterranean organisms. In the Yucatán Peninsula, such processes have been observed in cenotes of coastal areas (which constitute subterranean estuaries), such as Ox Bel Ha Cave System (Brankovits et al. 2017). In cenote Bang belonging to this system, bacterial mats are capable through methanotrophy, heterotrophy, and chemoautotrophy to make available organic compounds from methane and dissolved organic carbon which originated in the surface. The resulting products constitute the main food source for anchialine fauna (Brankovits et al. 2017).

In X-Batún, although bacterial structure, chemoautotrophy and subproducts have not already been quantified, the ecological pattern observed in benthic taxa i.e., the highest abundance in aphotic zone, suggests that there may exist interactions between bacterial activity and the benthic community. Similar patterns of benthic species distributions were observed in other freshwater cenotes of the Ring of Cenotes, such as Xoch, Tzitzila and Dzontila. There, the presence of gastropods in the light limited or aphotic zones was assumed as the result of interaction with chemolithotrophic bacteria or organic products resulting from chemolithotrophic processes (Grego et al. 2019). These biological patterns observed at regional scale in the Peninsula therefore, highlights that the bacterial and subterranean species relationships observed in subterranean estuaries such as in Ox Bel Ha, may be widespread in Yucatán and occurring as well in freshwater cenotes.

We hypothesized that the unique presence of epigeal ostracodes in the aphotic zone of X-Batún represent an ecological adaptation, resulting from active movements of the species from highly competitive zones in the surface to food rich and low competitive environment in the aphotic zone.

The SIMPER test revealed that the groups with higher statistical contribution to outer cenote zones, surface and open waters, were testate amoebae, copepods and mites. These taxa commonly inhabit lake environments and particularly zones where sun light is fully available, and primary productivity is high (Sigala et al. 2016; Charqueño et al. 2019; Montes-Ortiz and Elías-Gutiérrez 2020). This illustrates that surface waters of X-Batún have a biological structure typical of the epigeal environments. Comparatively, surficial fauna of X-Batún is similar to that from other oligotrophic cenotes in Yucatán Peninsula, except for the absence of ostracodes in surficial sediments (Smirnov and Elías-Gutiérrez 2011).

Influence of environmental variables of Cenote X-Batún over benthic assemblages

The measured environmental variables of cenote X-Batún show that there exists little variation from the upper layers to bottom. Light was the variable with more drastic changes as it varied from full incidence in surface and open waters to twilight in the cavern zone and complete darkness in the cave. Relatively homogeneous conditions in this system, can be expected, as it is primarily subterranean. Subterranean environments tend to be physically and chemically stable (Hüppop 2012). Particularly, in X-Batún, only a relatively little area (<0.1 ha) is interacting with the epigeal environments, and it is protected from wind and sun light by a dense canopy. Thus, drastic changes of water column physical or chemical conditions are not expected, even at surface waters. Linear correlation however, determined that light and TDS were the most influential for species assemblages. At regional scale, sun light is known as primary factor structuring biological compositions, because the full availability at surface is monotonically decreasing toward the deepest parts of the cave, changing the productivity and food webs (Culver and Pipan 2009; Hüppop 2012; Borisov et al. 2021). The DCA analysis, however, remarks that environmental variables exert different influences on the biological assemblages along the cave. The single entrance of the cenote, for example, characterized by full sun light incidence and high detritus input, support a typical epigeal benthic species assemblages associated to higher values of pH, temperature and, dissolved oxygen (Vinogradova and Riss 2007; van Hengstum et al. 2008; Pérez et al. 2013). Open water assemblage, featuring species from surface and deeper waters, seems to be little influenced by environmental variables, as it was located close to the center of the axes 1 and 2. This is expected as the variables are more stable in this zone, light is fully available and therefore autochthonous productivity seem to be the main source of food. The G3 and G4, corresponded to deeper zone of the cenote show a negative correlation with most of the environmental variables, as the values of most of them decrease. Positive correlations were recovered from conductivity and TDS. These species assemblages are similar in structure and mainly associated to zones where sun light is limited or lacking. In the NMDS, given the patterns of species assemblages and their influence by environmental variables, we deduce that sun light is the main driver for species distribution in X-Batún, which is also supported by linear correlation.

Conclusions

There is only scattered information available about the diversity, distribution patterns, colonization methods and ecology of the benthic assemblages in subterranean realms of the Yucatán Peninsula. We characterized the benthic fauna of a Yucatán water-filled doline and its submerged cave. The fauna was composed of eight higher taxonomic groups and four assemblages. The deepest, fully dark zones of the cave displayed the highest species diversity and abundance, which raises the possibility of chemoautotrophy. Environmental variables of the different zones of the water column display a little variation from the upper layers to bottom. Linear regression demonstrated that light gradient is the most relevant for species assemblages. Temperature, pH, dissolved oxygen, and light were the most meaningful for individual assemblage as evidenced by the DCA. This study highlights the importance of environmental gradients in structuring benthic biological communities in subterranean systems and the existence of complex biological interactions mediated by different sources of energy in the Yucatán Peninsula.

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